Neuron, Vol. 20, 825–828, May, 1998, Copyright ©1998 by Cell Press

Two for T

Minireview

Bruce P. Bean* and Stefan I. McDonough
Department of Neurobiology
Harvard Medical School
Boston, Massachusetts 02115

In 1981, Llinás and Yarom found that inferior olivary neurons hyperpolarized from rest could generate calcium-dependent action potentials that had even lower thresholds than sodium action potentials. To account for this, they proposed the existence of a "low threshold" calcium conductance that is largely inactivated at the resting potential. Several years later, Carbone and Lux (1984) made patch-clamp recordings in sensory neurons of a calcium current with exactly the properties inferred by Llinás and Yarom: activation by small depolarizations, rapid inactivation, and substantial steady-state inactivation at normal resting potentials. Almost simultaneously, a number of groups studying a variety of excitable cells, including neurons, cardiac muscle, and endocrine cells, characterized similar channels, variously called low threshold, low voltage–activated, and T-type channels. At the single channel level, T-type channels have a lower unitary conductance (~8 pS) than other calcium channels. At the whole-cell level, T-type currents are most obviously distinct from other calcium currents by their rapid inactivation (Figure 1A) and requirement for strongly negative holding potentials for full availability. The voltage dependence of activation of T-type current varies, and in some neurons the difference from "high voltage–activated" current is not dramatic (e.g., Figure 1C).

After the recognition of distinct low voltage–activated calcium channels in neurons, attention shifted to distinctions among different high threshold calcium channels, initially made by pharmacology and single channel characteristics. Then, channel by channel, molecular cloning defined the molecular basis of calcium current. Different cloned channels have now been identified more or less certainly with components of high voltage–activated current in native cells: α_{1S}, α_{1C}, and α_{1D} subunits with L-type channels in skeletal muscle, cardiac muscle, and neurons; α_{1B} subunits with N-type channels; α_{1A} subunits with both P-type and Q-type neuronal channels; and α_{1E} subunits with R-type channels (reviewed by Dunlap et al., 1995). Until now, however, T-type calcium channels were notably missing from the roster of cloned channels. Although it was initially suggested that the α_{1E} clone corresponds to T-type channels (Soong et al., 1993), careful comparison has shown qualitative differences (Randall and Tsien, 1997).

Now, two papers offer very different views of the molecular basis of T-type current. One is the straightforward and exciting discovery of a new cDNA whose expression produces channels with properties matching those of T-type channels (Perez-Reyes et al., 1998). The other is more unexpected: a report that the already-known α_{1B}, α_{1C}, and α_{1E} clones can produce single channel activity with many similarities to T-type channels (Meir and Dolphin, 1998).

Perez-Reyes and colleagues identified the new clone by screening GenBank expressed sequence tags (ESTs) for homology to existing α_1 subunits. The new channel α_1 subunit, named α_{1G}, has relatively weak overall homology to the high voltage–activated subunits but has important similarities in two regions likely to be crucial for calcium channel function: the "P region" of each S5–S6 linker, believed to be important for ion permeation and selectivity, and the S4 regions thought to underlie voltage-dependent gating. Mutation studies on L-type channels have shown that calcium selectivity depends on

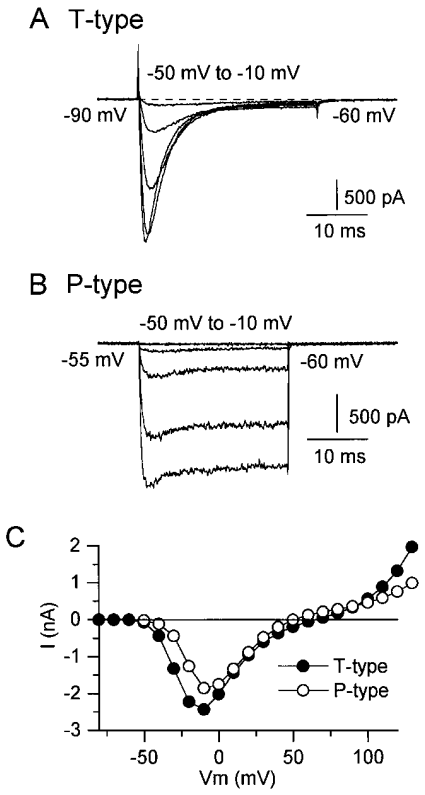

Figure 1. Kinetics and Voltage Dependence of T-Type and P-Type Calcium Currents in Mouse Cerebellar Purkinje Neurons

(A) T-type current isolated by blocking L-type current with 1 μM nimodipine and P-type and N-type currents with 10 μM ω-conotoxin MVIIC.

(B) Mainly P-type current (in a different cell) elicited from a holding potential of −55 mV (where T-type current is inactivated) in the absence of any blocker.

(C) Voltage dependence of peak current for the cells in (A) and (B). Both sets of currents recorded at 35°C. External solution (in mM): 160 TEACl, 5 $BaCl_2$, and 10 HEPES (pH 7.4) with 600 nM tetrodotoxin. Internal solution (in mM): 56 CsCl, 68 CsF, 2.2 $MgCl_2$, 4.5 EGTA, 9 HEPES, 4 MgATP, 14 creatine phosphate, and 0.3 GTP (pH 7.4). Corrected for linear leak and capacitative currents using current elicited by a scaled 10 mV hyperpolarizing pulse.

*To whom correspondence should be addressed.

four crucial glutamate residues, one in each P region (Ellinor et al., 1995). In α_{1G}, two of these are glutamate and the other two are aspartate. The difference could plausibly be related to the smaller single channel conductance of T-type channels. Intriguingly, the reversal potential for α_{1G} expressed in oocytes (+30 mV) was significantly less than for α_{1E} under the same ionic conditions (+70 mV). This suggests that α_{1G} channels may have less selectivity for barium versus potassium than do α_{1E} channels. However, contaminating currents may complicate the determination of reversal potential in oocytes, and native T-type channels have so far appeared to have divalent-versus-monovalent selectivity equivalent to other calcium channels (e.g., Figure 1C).

The previously known cloned calcium channels are believed to function as complexes of the main pore-forming subunit (α_1) along with two accessory subunits (β and α_2-δ) that are necessary for maximal expression with normal kinetics and voltage dependence (see Dunlap et al., 1995). However, coinjection of auxiliary subunits was not required for normal expression of the α_{1G} channels.

Before the discovery of the α_{1G} clone, many speculated that T-type channels might turn out to be more closely related to voltage-dependent sodium channels than to other calcium channels. One reason for this speculation is that the kinetics of T-type channels are similar to those of sodium channels, especially in inactivation, which is rapid, nearly complete, and highly voltage dependent at the macroscopic level (Figure 1A). The speculation was incorrect, since the α_{1G} sequence is less related to sodium channels than to other calcium channels (Tsien, 1998). Nevertheless, it can be expected that the molecular determinants of T-type channel inactivation will be an early focus of work with the new clone, guided by the analyses of the other fast inactivating channels, sodium currents and A-type potassium channels, whose mechanisms of inactivation seem quite different from each other.

The paper by Meir and Dolphin challenges the idea that T-type channels are necessarily a class distinct from high voltage–activated channels. They report that α_{1B}, α_{1C}, or α_{1E} channels expressed in COS cells produce low conductance single channel events as well as the expected higher conductance events for each channel type. The small conductance openings do not appear to result from a distinct type of channel (e.g., endogenous T-type channels whose expression is enhanced by the heterologously expressed channels), because the occurrences of low conductance and high conductance openings were not independent. In particular, low conductance openings were always present in patches that had high conductance openings, and many patches had neither. This does leave open the possibility that the conductances come from distinct channel types that are always colocalized because of common pathways of membrane insertion or anchoring. However, the simplest interpretation is that the low conductance openings arise from the same channels underlying the high conductance openings. The intriguing feature of the low conductance openings is that they have many properties corresponding to T-type channels. In particular, low conductance openings are prevalent at small depolarizations and, when averaged, yield inactivating currents. Perhaps most strikingly, average currents also have slow deactivation, very similar to T-type currents (which, for comparison, the authors recorded under the same conditions in NG108–15 cells).

The most convincing evidence that the low conductance and high conductance openings arise from the same channels would be observation of both, in a mutually exclusive manner, in a patch unambiguously containing only one channel. One patch with an α_{1B}/α_2-δ/β_{2a} combination appeared to meet this condition, but it is hard to be sure that only a single channel was present since neither low conductance nor high conductance openings occurred with a high probability, and at voltages sufficiently depolarized to significantly activate high conductance events, low conductance events were difficult to distiguish from baseline noise.

Meir and Dolphin found that low conductance openings are more prominent when α_1 subunits are expressed alone, without β or α_2 subunits. Thus, they raise the possibility that native T-type channels may consist of α_1 subunits without associated auxiliary subunits, as well as the possibility that conversions between low conductance and high conductance openings involves reversible, voltage-dependent association of α_1 with accessory subunits.

A number of loose ends are left by this intriguing report. If T-type current arises from the same α_1 subunits that underlie high threshold current, it might be expected to have similar pharmacology. (Although coexpression of the accessory subunits can alter antagonist potency, the binding sites for the canonical blockers of L-type and N-type currents are on the α_1 subunits.) This was not examined in the heterologous system. In native cells, most studies show distinct pharmacology of T-type channels, although in some cases sensitivity to blockers of high voltage–activated channel types has been reported. If T-type current does correspond to "naked" α_1 subunits, it might be expected to be even more sensitive to inhibition by transmitters that act by G protein $\beta\gamma$ subunits, whose binding site may partially overlap with that of calcium channel β subunits (De Waard et al., 1997). Interestingly, transmitter inhibition of apparent T-type current has occasionally been seen in neurons, although it seems uncommon. Treatment of cultured cells with antisense RNA for calcium channel β subunits makes the calcium current more sensitive to transmitter modulation (Berrow et al., 1995), but the currents do not seem to become more T-like in kinetics or voltage dependence under these circumstances.

Other interpretations can be given of the low conductance openings seen by Meir and Dolphin. These openings tend to occur at smaller depolarizations and, at a given voltage, at earlier times than the high conductance openings. They could represent openings of partially activated channels, similar to those recently reported for Shaker K^+ channels (Zheng and Sigworth, 1997). Another possibility is that the low conductance openings represent channels modulated in some way, although channels modulated by G proteins might be expected to require larger, not smaller, depolarizations to open. Whatever the interpretation, it remains to be seen whether low conductance openings of high voltage–activated channels are a common occurrence in native cells or in

other expression systems. Such openings might often be overlooked or ignored. However, several recent studies of transmitter modulation of native and cloned α_{1B} channels specifically searched for and failed to detect low conductance openings (Carabelli et al., 1996; Patil et al., 1996).

Clearly, the widespread expression in the brain of the clone identified by Perez-Reyes and colleagues, and the close correspondence of its expressed currents with native T-type currents, make it seem very unlikely that low conductance openings of other α_1 subunits account for T-type current in most cases. Nevertheless, the heterogeneity of the pharmacology and kinetics of low voltage–activated current among cell types (Huguenard, 1996) leaves open the possibility that such openings underlie some instances of T-type current. This possibility should spur more detailed examination of the properties, especially the pharmacology, of low voltage–activated current. Heterogeneity of T-type current may also reflect the possible existence of other genes related to α_{1G} or alternative splicing of gene products.

The new attention to the molecular nature of T-type channels should lead to progress in understanding their functions, which are still largely unclear. T-type channels are present in a variety of excitable cells that show spontaneous activity, including sinoatrial nodal cells of the heart, neuroendocrine cells, and thalamic neurons. In most cases, however, there is no direct evidence that current through T-type channels is crucially involved in pacemaking. It is possible that spontaneous activity in some cells occurs at voltages where T-type channels are almost completely inactivated throughout a firing cycle. A better-documented function in neurons is to confer the striking property of firing action potentials after hyperpolarization (see Huguenard, 1996). Hyperpolarization removes inactivation of T-type channels, and in some neurons return to the resting potential is sufficient to activate the channels and produce a calcium spike, often with superimposed sodium spikes. The result is that a purely hyperpolarizing input from presynaptic "inhibitory" neurons can excite a cell by triggering rebound firing of action potential bursts. This behavior is crucial for the synchronized, oscillatory firing of thalamic neurons related to spindle waves.

Some of the functions of T-type channels may not involve firing of action potentials at all but rather mediation of steady inward current at a cell's resting potential. Such steady currents are possible since channels can apparently open at voltages where inactivation is still incomplete. Although channel open probability is likely to be very low, the resulting influx of calcium can be significant, since it is steady and the driving force is high. In adrenal glomerulosa cells, calcium-dependent secretion of aldosterone apparently depends on the steady influx of calcium through T-type channels at a steady resting potential, rather than action potential activation of brief calcium currents (Cohen et al., 1988). With this mechanism, small changes in resting potential can regulate calcium entry by changing the degree of activation or inactivation. Mediating steady entry of calcium may be the predominant function of T-type channels when they occur in cells that do not fire action potentials, such as fibroblasts (Chen et al., 1988). There

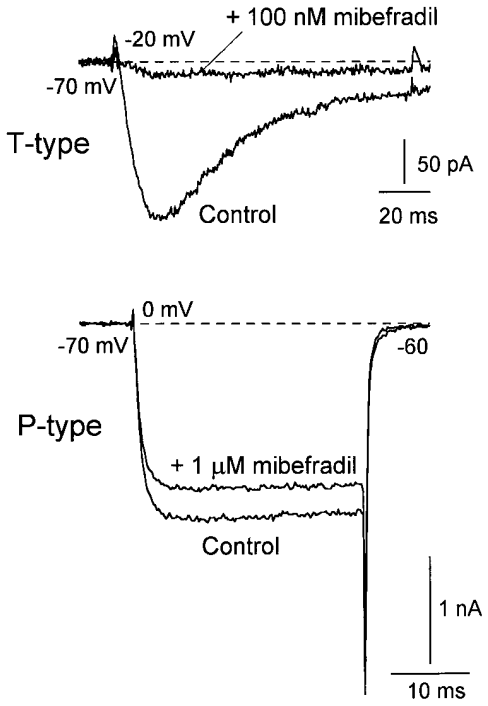

Figure 2. Sensitivity to Mibefradil of T-Type and P-Type Current in Rat Cerebellar Purkinje Neurons

Solutions as in Figure 1, except that P-type current was recorded in the presence of 1 μM nimodipine to block L-type current. Recorded at 22°C. Mibefradil was the kind gift of Dr. J.-P. Clozel (Hoffmann-LaRoche, Basel).

is increasing evidence suggesting that expression of T-type channels may be associated with cell growth and proliferation in response to growth factors (e.g., Xu and Best, 1990).

Neither of the two papers on the molecular basis of T-type current includes pharmacology, illustrating a major factor that has impeded the understanding of the functions of T-type channels: the lack of a potent and selective blocker. The new clone should help stimulate efforts to develop better pharmacological agents. Many studies have used sensitivity to block by nickel ions to identify T-type current, but selectivity over other channel types is relatively poor, and potency depends on ionic conditions. Many organic molecules block T-type channels, but most also block various high threshold channels. Of these, the most selective is the antihypertensive mibefradil, which blocks T-type channels in NG108–15 cells with an EC_{50} (at −80 mV) of ~1 μM compared to ~3 μM for L-type, N-type, and P-type channels (Bezprozvanny and Tsien, 1995; Randall and Tsien, 1997). Because block of all types of channels is more potent when channels are partly inactivated and T-type channels are mostly inactivated at resting potentials, the degree of selectivity can be quite high at physiological resting potentials (Figure 2).

A particular reason for interest in the pharmacology of T-type channels in the CNS is the inhibition of the channels by a number of anticonvulsant drugs, especially petit mal drugs such as ethosuximide. Interestingly, sensitivity to ethosuximide is dramatically different

for T-type channels in different neurons, one indication that there are multiple types of T-type channels (Huguenard, 1996). T-type calcium current is increased in reticular thalamic neurons in a rat model of absence epilepsy (Tsakiridou et al., 1995). It will be very interesting to determine whether α_{1G} channels are involved in epilepsy and whether they are targets of anticonvulsants.

Selected Readings

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